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English version

Standardized scaffolds library for tissue engineering research and industrial applications

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Ref. No.:CWA 18130:2024 E

Contents

Page

European foreword

This CEN Workshop Agreement (CWA 18130:2024) has been developed in accordance with the CEN-CENELEC Guide 29 “CEN/CENELEC Workshop Agreements – A rapid prototyping to standardization” and with the relevant provisions of CEN/CENELEC Internal Regulations — Part 2. It was approved by a Workshop of representatives of interested parties on 2024-05-31, the constitution of which was supported by CEN following the public call for participation made on 2023-10-30. However, this CEN Workshop Agreement does not necessarily include all relevant stakeholders.

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Introduction

Tissue engineering, regenerative medicine and biofabrication pursue the objective of reconstructing or recreating damaged or lost tissues by providing cells with the adequate synthetic biomimetic nano-, micro- and macro-environments to let them deploy their healing potential. To this end, an increased number of combinations of bioinspired geometries, biomaterials, and manufacturing technologies, in many cases assisted by biomolecules, growth factors and drugs, have been researched and developed during the last decades. These efforts have led to literally thousands of artificial cell niches, extracellular matrices and scaffolds for tissue engineering, which act as enhanced implants or as advanced therapy medicinal products that support cells during healing and regeneration. In fact, since the dawn of tissue engineering, scaffolds have played a fundamental role as structural biomaterials units that support cell attachment, proliferation, and differentiation into relevant tissues.

As enabling technologies for the materialization of design-controlled tissue engineering scaffolds it is important to concentrate on additive manufacturing. Additive manufacturing technologies (AMTs) have emerged in the last decades, as highly transformative resources, enabling solid freeform fabrication, fostering freedom of design, promoting the personalization of devices and nurturing the world of design with reformulated guiding principles. In fact, AMTs have reshaped several industrial fields and, in connection with the “maker movement” or “maker culture”, led to the democratization of technology. The geometrical complexity achievable through AMTs, which can be employed for the integration of functionalities, helped to set the foundations of tissue engineering as a field. To cite some examples, fused deposition modelling of polymers and composites, selective laser sintering and melting of alloys, laser stereolithography and digital light processing of photopolymers, lithography-based ceramic manufacturing, or inkjet printing of hydrogels (as precursor of bioprinting), are among the AMTs employed in the last couple of decades to create biomimetic tissue engineering scaffolds.

Indeed, tissue engineering, regenerative medicine and biofabrication may benefit from progressively implementing and spreading the use of internationally accepted and applied standards, conceived to support the repeatability, replicability and efficient comparing of research results among researchers and practitioners worldwide.

Considering that scaffolds play a fundamental role in most tissue engineering procedures, it would be interesting to count with an internationally accepted set of geometries, acting as a library of lattices, porous materials and scaffolding structures, which could be employed for comparative purposes among materials and technologies under development. To this end, an open-source library of additively manufactured tissue engineering scaffolds has been recently implemented (Martínez Cendrero, A.; Franco Martínez, F.; Solórzano Requejo, W.; Díaz Lantada, A.- “**Open-source library of tissue engineering scaffolds**”, Materials and Design, 223, 111154, 2022), which constitutes a fundamental reference document for this document.

This library stands out for providing a comprehensive collection of scaffolds’ designs, implemented considering the specific features of most additive manufacturing technologies applicable to tissue engineering, regenerative medicine and biofabrication.

The focus on additively manufactured scaffolds is essential, due to the impact of AMTs in the tissue engineering and biofabrication fields and because these technologies provide geometrical control from the design stage and extreme materials versatility, as polymers, alloys, ceramics, composites, biological and even engineered living materials can be additively processed. In addition, AMTs enable multi-material, multi-scale and multi-phase bioinspired or biomimetic scaffolds, which from the point of view of regenerative medicine proves fundamental. However, a systematic approach to the engineering design of these bioinspired geometries, considering both the topology of the porous scaffolding lattices or networks and their surface topography, is still required.

Consequently, the goal of this document is to define a set of widely accepted additively manufactured scaffolds geometries and related documentation for supporting researchers, industries and clinical practitioners in the areas of tissue engineering and regenerative medicine, both in design, manufacturing, selection and evaluation tasks.

1 Scope

This document defines a set of geometries for additively manufactured tissue engineering scaffolds conceived for being printed employing all the standard families of additive manufacturing technologies according to the standards EN ISO 17296-2:2016 and EN ISO/ASTM 52900:2021.

This document provides a systematic engineering design and selection methodology for additively manufactured tissue engineering scaffold. Methods for their methodical testing and comparison, to take account of the processable materials properties and features of the AMTs employed for their creation and to verify their effects on final results, are provided.

Accordingly, this document is intended to be of interest for tissue engineering scaffolds designers and manufacturers, for designers and manufacturers of scaffold-inspired implants and for healthcare professionals in the fields of tissue engineering, regenerative medicine and biofabrication.

2 Categorization of tissue engineering scaffolds

Seven main families of additive manufacturing technologies are able to process most materials applicable in medical practice and can lead to tissue engineering scaffolds with different morphologies, precision, resolution and interesting features. In short, photopolymerization, material extrusion, material jetting, binder jetting, powder bed fusion, direct energy deposition and sheet lamination are the families, all of which count with several technological materializations within. Design for additive manufacturing (DfAM) strategies consider the features of the different families and processes and provide geometries for achieving the best possible geometries.

This DfAM approach is of pivotal importance for tissue engineering scaffolds, which in many cases bring these technologies and the related processable materials to their limits and beyond.

To cite some examples, when designing for photopolymerization the pixel dimensions of the digital light processing systems used must be considered, while the extrusion diameter proves fundamental when designing for fused deposition modeling. Besides, when applying laser powder bed fusion, the printable design varies depending on the applicable post processes (f.e. chemical etching or electropolishing) or their absence if the part is employed as printed. Furthermore, some technologies always require support materials, which are removed after printing, unless special design guidelines are followed, while others lead to self-supported parts and avoid or minimize such post-processing and related debris generation.

Currently, published studies linked to the design, fabrication and in vitro, ex vivo or in vivo application of tissue engineering scaffolds employ literally thousands of geometries, materials, manufacturing technologies and combinations thereof, which makes comparability and even repeatability and reproducibility of results challenging. This brings to non-straightforward development processes when approaching the engineering design of innovative tissue engineering scaffolds capable of taking benefit of being additively manufactured. In addition, the industrial applicability and the societal transfer are hurdled by the lack of standardized geometries and systematic selection processes.

In agreement with the commonly employed scaffolding geometries widely available in the literature and considering the main families of additive manufacturing technologies normally employed for the creation of both mono- and multi-material tissue engineering scaffolds, the following main categories for tissue engineering scaffolds are proposed:

— Mono-material scaffolds based on periodic unit cells or lattices inspired by Bravais.

— Mono-material scaffolds based on woodpile structures.

— Mono-material scaffolds based on multi-scale fractal lattices.

— Multi-material scaffolds based on combined lattices for graded designs.

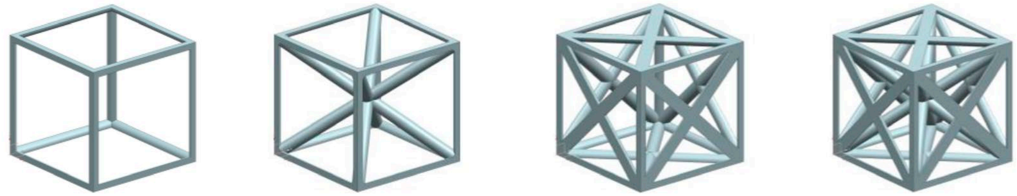
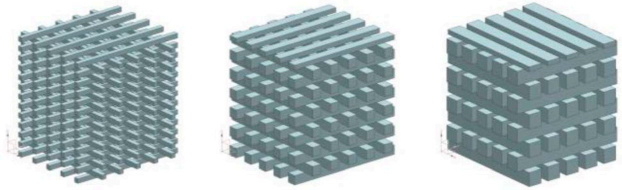
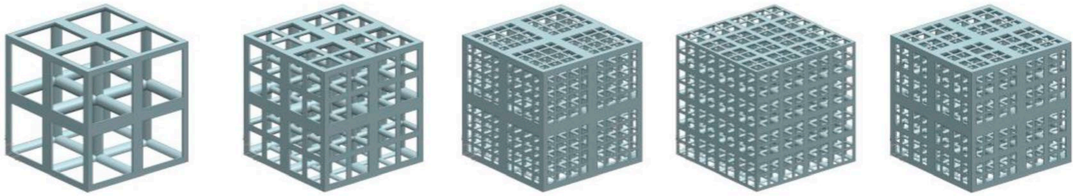
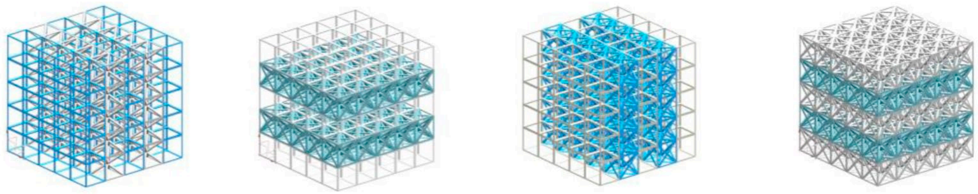
— Multi-material scaffolds based on combined unit cells at the same scale.

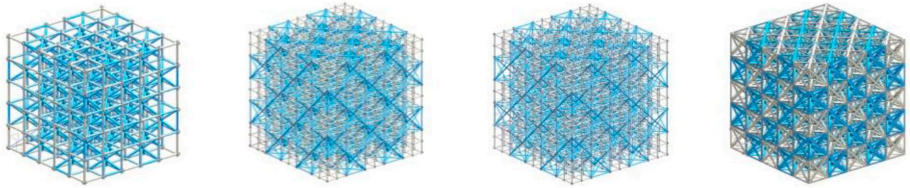
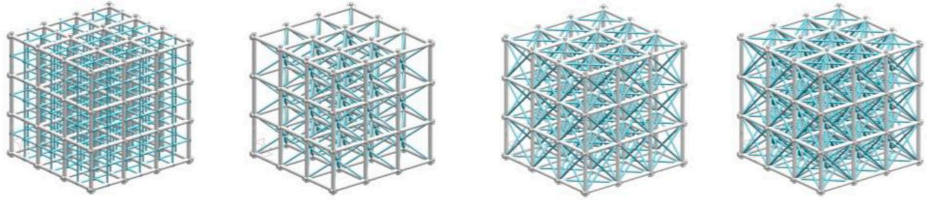
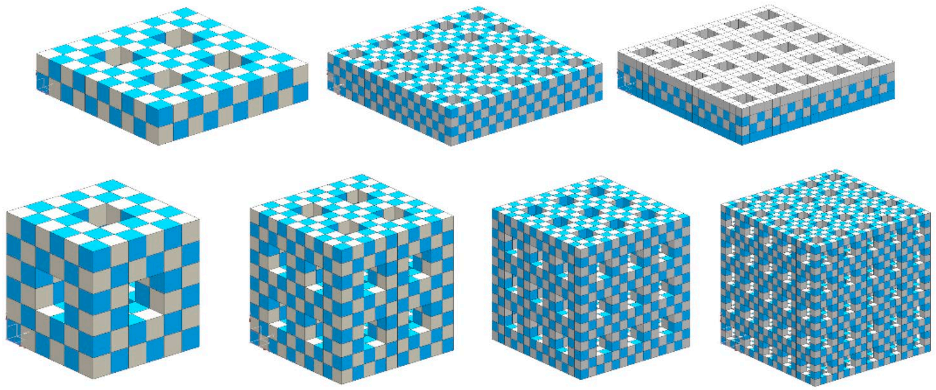
— Multi-material scaffolds based on combined unit cells at different scales.

— Multi-material scaffolds based on pixelated or voxelated arrangements of matter.

Accordingly, Table 1 presents a collection of geometries for tissue engineering scaffolds, grouped by the listed families or categories, including mono-material and multi-material solutions, and a description of the applicable AMTs for the materialization of the different families or categories of tissue engineering scaffolds. DfAM principles have been applied for their implementation.

Table 1 — Summary of geometrical families with selected examples from the open-source library of tissue engineering scaffolds. *Suboptimal technology for printing the selected geometries (other geometries may work better). **Better avoided although printable. Acronyms: FDM – fused deposition modeling, SLA – laser stereolithography, DLP – digital light processing, LCM – lithography based ceramic manufacture, LMM – lithography-based metal manufacture, SLS – selective laser sintering, SLM (PBLF) – selective laser melting / (powder bed laser fusion), 2PP – two photon polymerization.

Materials	Geometries	Selected images as examples from the OS library
Mono-material scaffolds	Periodic unit cells or lattices inspired by Bravais	
	Woodpile structures	
	Multiscale / fractal lattices	
Multimaterial (MM) scaffolds	Combined lattices for graded designs	

Materials	Geometries	Selected images as examples from the OS library
	Combined unit cells with the same scale	
	Combined unit cells at different scales	
	Pixel- or voxel-based designs	

3 Definition of scaffolds design selection methods

Personalized implants design is addressed in the CWA 18131:2024, *Workflow from medical images towards optimal personalized implant designs*. Personalized implants for tissue engineering benefit in many cases from the conformal mapping of selected tissue engineering scaffolds across the whole working volume of the implant. Hence, according to patients' medical images, bulk implants may be designed, which are subsequently rendered porous for enhanced biomechanical performance and biological response, through the application of scaffolding lattices to the bulk volume.

To select the most adequate tissue engineering scaffolds for being mapped across the personalized implant, it is important to consider the mechanical properties of the original extracellular matrix(es) or tissue(s) being repaired, the available manufacturing technologies and usable materials, the need for special fluidic interactions that may improve cellular colonization, vascularization and long-term viability, among others. Considering the vast portfolio of available additive manufacturing technologies and materials, the use of Ashby charts for materials selection purposes is advisable. In connection with the proposed library of scaffolding materials and categories for tissue engineering scaffolds, Asby diagrams for selected geometries of Table 1 are already available (Martínez Cendrero, et al. 2022; Open-source library of tissue engineering scaffolds: https://zenodo.org/record/6387574#.YkGd5OdBxPY). These include stiffness vs relative density graphs, compressive Young's moduli vs shear moduli, exposed surface vs relative density and pressure drop across the scaffold vs flow speed.

To further expand the collection and make it more versatile, as well as usable, it is necessary to characterize the employable geometries and to represent their properties in an adimensional or comparable way, so as to progressively expand the available Ashby diagrams.

The selection methodology for tissue engineering scaffolds is schematically outlined in Figure 1 and illustrated through two selection examples of tissue engineering scaffolds for bone and cartilage repair.

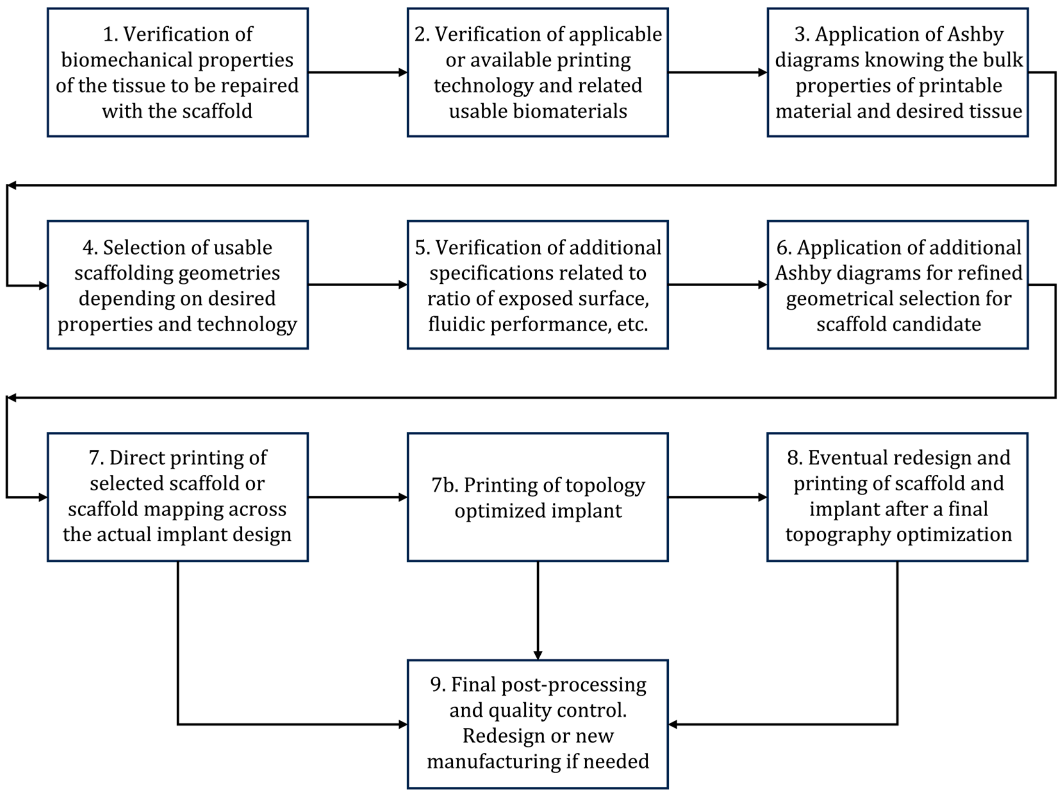
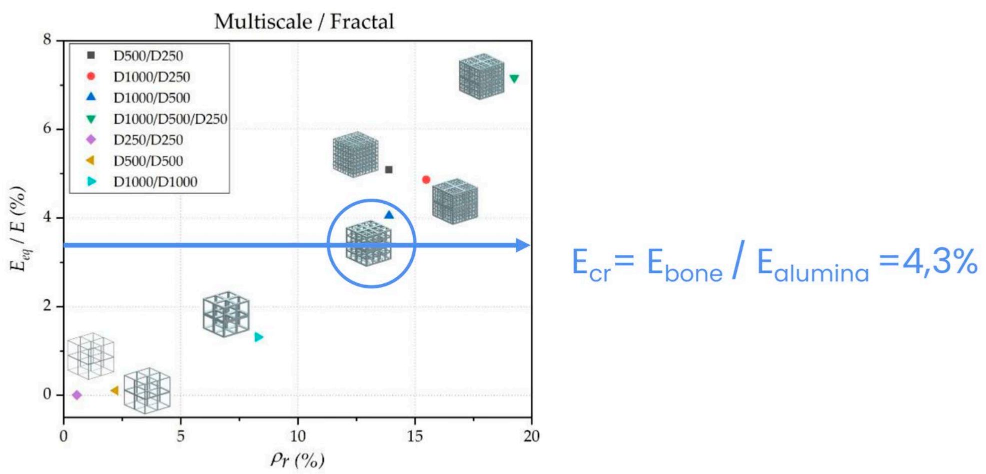


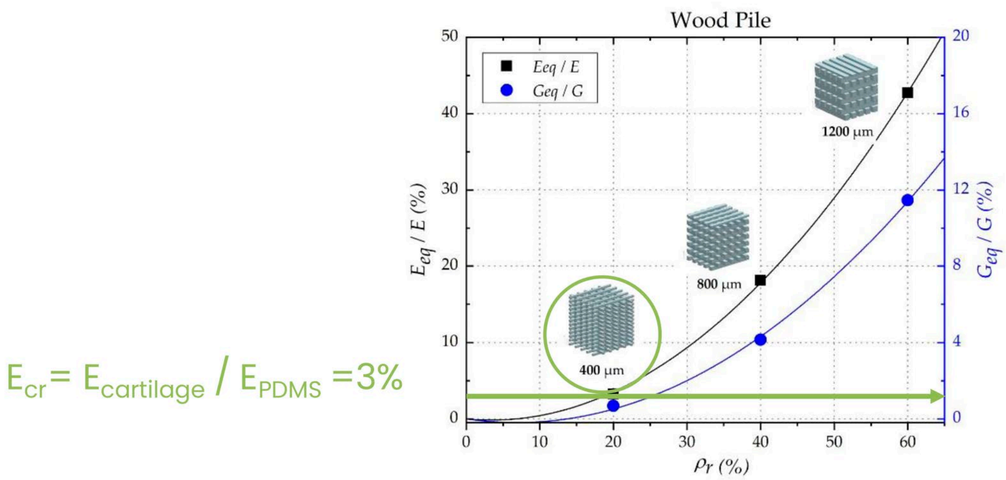
Figure 1 — Schematic guideline for the selection and application of standardized tissue engineering scaffolds created using additive manufacturing technologies

First usability case (Figure 2a) deals with the selection of a scaffold geometry for bone repair to be printed by lithography-based ceramic manufacturing using alumina. A multi-scale scaffold may be desired due to the functionally graded nature of bone. From the Ashby diagrams available in the collection (Martinez Cendrero et al. 2022) the one relating compression modulus and relative density for multiscale / fractal scaffolds is chosen. Relative compression modulus of 4.3% is calculated by dividing the modulus of bone (15 GPa) by the one of bulk alumina (350 GPa). With this value the most interesting geometry from a mechanical perspective is chosen.

Second usability case (Figure 2b) selects a geometry for cartilage repair. In this case, polydimethylsiloxane (PDMS) is to be bioprinted or deposition modelled as liquid. In this case, common woodpile structures may be usable. The desired compression modulus for cartilage is 0.3 MPa, which divided by the c.a. 10MPa of PDMS modulus gives us a relative value of 3%, which leads to a potentially useful scaffold.



a) Selection of a scaffold geometry for bone repair to be printed by lithography-based ceramic manufacturing using alumina



b) Selection of a scaffold geometry for cartilage repair to be printed using bioprinting or liquid deposition modeling of PDMS

Figure 2 — Usability of Ashby diagrams for scaffolds selection purposes

Bibliography

Reference documents and already available geometries:

[1] Martínez Cendrero, A.; Franco Martínez, F.; Solórzano Requejo, W.; Díaz Lantada, A. — "Open-source library of tissue engineering scaffolds. *Mater. Des.* 2022, **223** p. 111154

[2] Open-source library of tissue engineering scaffolds: **.**zenodo.org/record/6387574#.YkGd5OdBxPY

[3] EN ISO 17296-2:2016, *Additive manufacturing — General principles — Part 2: Overview of process categories and feedstock (ISO 17296-2:2015)*

[4] EN ISO/ASTM 52900:2021, *Additive manufacturing — General principles — Fundamentals and vocabulary (ISO/ASTM 52900:2021)*